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Investigation on the interaction of pyrene with bovine serum albumin using spectroscopic methods

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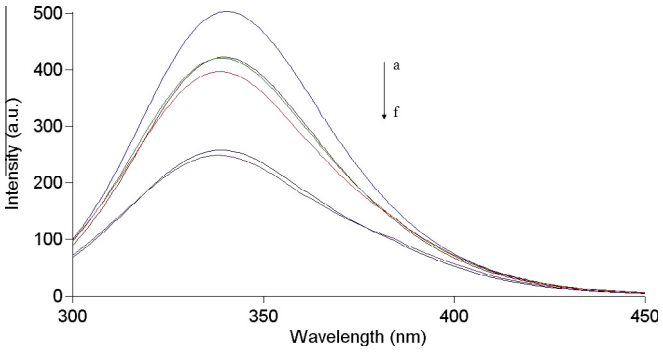
HIGHLIGHTS

- The fluorescence quenching of bovine serum albumin (BSA) by pyrene is static.
- The pyrene–BSA complex has been formed.
- The energy transfer of BSA to pyrene occurs.
- Van der Waals forces and hydrogen bonds play major roles in the binding process.
- The conformational changes indicate that pyrene induces damage to BSA.

GRAPHICAL ABSTRACT

The changes of FL spectra of pyrene–BSA intensity with pyrene concentration. $a = 0$; $b = 0.2 \times 10^{-5}$; $c = 0.4 \times 10^{-5}$; $d = 0.6 \times 10^{-5}$; $e = 0.8 \times 10^{-5}$; $f = 1.0 \times 10^{-5}$ mol L^{-1}.



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ABSTRACT

This paper was designed to investigate the interaction of pyrene with bovine serum albumin (BSA) under physiological condition by spectroscopic methods. Spectroscopic analysis of the emission quenching revealed that the quenching mechanism of BSA by pyrene was static. The binding sites and constants of pyrene–BSA complex were observed to be 1.20 and 2.63×10^6 L mol^{-1} at 298 K, respectively. The enthalpy change (ΔH) and entropy change (ΔS) revealed that van der Waals forces and hydrogen bonds stabilized the pyrene–BSA complex. Energy transfer from tryptophan to pyrene occurred by a FRET (fluorescence resonance energy transfer) mechanism, and the distance ($r = 2.72$ nm) had been determined. The results of synchronous, three-dimensional fluorescence, and circular dichroism spectra showed that the pyrene induced conformational changes of BSA.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants. PAHs in the environment arise principally from the combustion of fossil fuels, industrial emissions (e.g. coke oven plants, cement plants, incinerators, aluminum industries), mobile sources (e.g. automobile and aircraft exhaust) and forest fires [1]. Since the pyrene metabolites, 1-hydroxypyrene (1-OHP) and its glucuronide conjugate, are used as the most common biomarker for recent PAH exposures [2–4], and pyrene occurs at relatively high concentrations in PAH mixtures [5,6], this chemical is an important toxicological agent of major concern. However, the toxic-

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ity produced by it direct interaction with biological macromolecules in vivo has not been explored previously. Consequently, a thorough study on the mechanism of toxicity of the pyrene is needed.

It is well-known that the absorption, distribution, metabolism and excretion of various ligands are strongly affected by the protein–ligand interactions in plasma [7]. Serum albumin is the most abundant protein in the circulatory system. It possesses many physiological functions and plays a dominant role in drug disposition and efficacy [8,9]. The distribution and metabolism of a great number of biologically active compounds are dependent on their affinities toward serum albumin to a certain extent [10]. BSA was used in the interaction studies because of its stability, low cost, and unusual ligand-binding properties in this study. Furthermore, it displays approximately 76% sequence homology and a repeating pattern of disulfides with human serum albumin (HSA) [11,12].

In this work, investigation on the interaction between pyrene and BSA in vitro was reported using several spectroscopic techniques under physiological condition. The binding characteristics between pyrene and BSA were analyzed. The binding constants, binding sites along with thermodynamic parameters and the binding distance were estimated. The conformational changes of BSA were also explored.

Materials and methods

Materials

BSA was purchased from Sigma Chemical Co. (America) and used without further purification, its molecular weight was assumed to be 68,000 to calculate the molar concentrations. All BSA solutions were prepared in the Tris–HCl (pH = 7.4) buffer solution, and the BSA stocks solution (1.0×10^{-4} mol L^{-1}) was kept in the dark at 277 K. Pyrene was purchased from Fluka (Germany). All other reagents were of analytical grade and double distilled water was used throughout the experiment.

Apparatus and methods

All the fluorescence spectra were recorded on spectrofluorimeter Model F-7000 spectrophotometer (Hitachi, Japan) at 298 and 310 K, using 2.5 nm slit width. The excitation wavelength was 280 nm, and the emission spectra were recorded from 300 to 500 nm.

Synchronous fluorescence spectra were obtained by scanning simultaneously the excitation and emission monochromator. The wavelength interval ($\Delta\lambda$) is fixed individually at 15 and 60 nm, at which the spectrum only shows the spectroscopic behavior of tyrosine and tryptophan residues of BSA, respectively.

The three-dimensional fluorescence spectra were performed under the following conditions: the emission wavelength scan range was recorded between 240 nm and 440 nm, the excitation wavelength scan range was recorded from 200 to 500 nm at 2.5 nm increments. The other scanning parameters were just the same as the fluorescence quenching spectra.

Circular dichroism (CD) measurements were recorded on a MOS-450 (Bio-Logic, France) spectropolarimeter (200–250 nm and cell length path was 1 cm) by keeping the concentration of BSA constant (1.0×10^{-6} mol L^{-1}) while varying the pyrene concentration from 0 to 1.5×10^{-5} mol L^{-1}.

Results and discussion

The binding mechanism

Fluorescence spectroscopy is widely employed to study proteins and peptides. The intrinsic fluorescence of BSA is very sensitive to its microenvironment. Actually, the intrinsic fluorescence of BSA is almost contributed by tryptophan and tyrosine, because phenylalanine has a very low quantum yield [13].

Fluorescence quenching is the decrease of the quantum yield of fluorescence from a fluorophore induced by a variety of molecular interactions, such as excited-state reactions, energy transfer, ground-state complex formation and collisional quenching [14]. The different mechanisms of quenching are usually classified as either dynamic quenching or static quenching, which can be distinguished by their different dependence on temperature and viscosity, or preferably by lifetime measurements. Dynamic quenching and static quenching are caused by collisional encounters and ground-state complex formation between fluorophores and quenchers, respectively.

Fig. 1 shows the fluorescence emission spectra of BSA in the presence of different concentrations of pyrene at 298 K. When desired amount of pyrene was titrated into a fixed concentration of BSA, the fluorescence intensity of BSA decreased progressively, indicating that pyrene could quench intrinsic fluorescence of BSA through interacting with BSA. The fluorescence quenching was usually analyzed using the well-known Stern–Volmer equation [15–17]:

$$F_0/F = 1 + \mathrm{Ksv}[Q] = 1 + K_q\tau_0[Q] \quad (1)$$

where F_0 and F represent the fluorescence intensity in the absence and presence of quencher (pyrene), respectively. K_q is the quenching rate constant of the biomolecule, Ksv is the Stern–Volmer quenching constant, [Q] is the concentration of quencher and τ_0 is the average life-time of the biomolecule without quencher. As the average fluorescence lifetime of biopolymer is about 10^{-8} s, according to the Stern–Volmer equation and our experimental results, the bimolecular quenching constant (K_q) which we calculated was 3.53×10^{13} - L mol^{-1} s^{-1}. In general, maximum collisional quenching constant (k_q) of various kinds of quenchers to biopolymers is 2.0×10^{10} - L mol^{-1} s^{-1} [18,19]. But for BSA-pyrene system higher quenching rate constant (3.53×10^{13} L mol^{-1} s^{-1}) was obtained. This showed that the quenching of BSA by pyrene was static in nature. Therefore, it depends on the formation of complex between pyrene and BSA.

Binding constants and binding sites

Fluorescence quenching of BSA in the presence of various concentrations of pyrene can be analyzed to obtain various binding parameters according to the following equation:

$$\lg\left(\frac{F_0 - F}{F}\right) = \lg K + n \lg[Q] \quad (2)$$

We changed the equation as follow:

$$\lg\left(\frac{F}{F_0 - F}\right) = n \lg[Q]^{-1} - \lg K \quad (3)$$

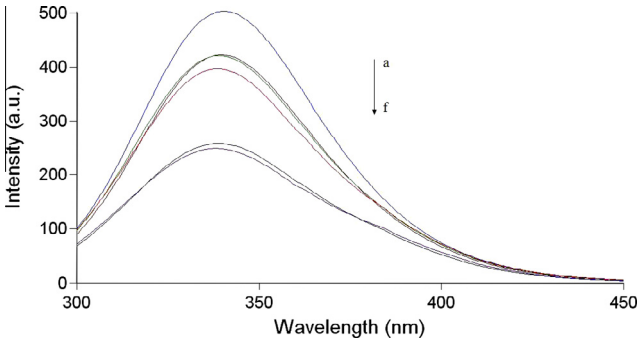

Fig. 1. The changes of FL spectra of pyrene–BSA intensity with pyrene concentration. $a = 0$; $b = 0.2 \times 10^{-5}$; $c = 0.4 \times 10^{-5}$; $d = 0.6 \times 10^{-5}$; $e = 0.8 \times 10^{-5}$; $f = 1.0 \times 10^{-5}$ mol L^{-1}.